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Pollination by long-proboscid flies in the endangered African orchid *Disa scullyi*

S.D. Johnson

School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa

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Abstract

Flowers specialized for pollination by long-proboscid flies are particularly prevalent in the southern African flora. Although many orchids in this region possess flowers with traits that are consistent with long-proboscid fly pollination, evidence from field observations is lacking for most of these species. Flowers of the critically endangered orchid *Disa scullyi* Bolus (Orchidaceae) were observed to be pollinated by the large nemestrinid fly species *Prosoeca ganglbaueri* Lichtwardt at a remote site in the Cape Drakensberg mountains, South Africa. The orchid's floral spur contains copious amounts of dilute sucrose-rich nectar and its length (c. 42 mm) closely matches that of the fly proboscis (c. 40 mm). Flies caught on the orchids carried pollinaria of *D. scullyi* on the basal portion of their proboscides. Although flies were not common at the study site, they showed considerable fidelity to *D. scullyi*, resulting in high levels of pollen removal and deposition in flowers in the population. Habitat loss and trampling by cattle, rather than pollination failure, appear to pose the greatest threat to the few remaining populations of this species.

Keywords: Conservation; *Disa*; Mutualism; Nemestrinidae; Orchidaceae; *Prosoeca* specialization

1. Introduction

The orchid genus *Disa* displays remarkable diversity in floral form, reflecting a process of pollinator-driven adaptive radiation (Johnson et al., 1998). A complete understanding of the patterns of floral evolution in this genus will require data on the pollination of all, or at least the majority of the species, but pollinators have been recorded for only one third of species in the genus to date. It is particularly difficult to document pollination systems of the rarer *Disa* species, some of which are on the verge of extinction.

Disa scullyi H. Bolus is one of the rarest southern African orchids and is currently listed as critically endangered (Golding, 2000). Collections have been made from seven populations in the Drakensberg mountains, but most of these populations seem to have been extirpated by farming activities in the past twenty years (Golding, 2000; SD Johnson, personal observation). The flowers of *D. scullyi* are pale pink in colour, long-spurred and unscented, traits that are found in many plants pollinated by long-proboscid flies (Goldblatt and Manning, 2000). Other *Disa* species with similar traits, such as *D. oreophila* H. Bolus subsp. *erecta* H.P. Linder, *D. pulchra* Sond., *D. draconis* (L.f.) Sw. and *D. harveiana* Lindl. have been shown to be pollinated by these flies (Johnson and Steiner, 1995, 1997; Johnson, 2000). It is now well-documented that long-proboscid flies belonging to the families Nemestrinidae and Tabanidae form one of the most important elements of the pollinator fauna in southern Africa and would have played an important role in floral diversification in the region (Vogel, 1954; Johnson and Steiner, 1997, 2003; Manning and Goldblatt, 1997; Goldblatt and Manning, 2000; Goldblatt et al., 2001).

The aims of this study were to document the pollination system of *D. scullyi* in order to shed light on floral evolution in the species and also, because of its endangered status, to better understand its habitat requirements in terms of pollinators.

2. Materials and methods

2.1. Study population

Observations were carried out in January 1993, 1994, 2001 and 2005 in a population of approximately 250 plants of

E-mail address: Johnsonsd@ukzn.ac.za.

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D. scullyi in a wetland on the "Bastervoetpad" pass (27°59′ S 31°10′ E) in the Drakensberg range of the Eastern Cape province of South Africa. This population, located at 2200 m, is probably the largest remaining of this species. Plants grow in grass tussocks separated by water rivulets in well-drained marshland. They produce an inflorescence 20–50 cm in height with up to 20 rose pink flowers. A voucher specimen from this population was deposited in the Bews Herbarium (NU) at the University of KwaZulu-Natal, Pietermaritzburg.

2.2. Floral traits

Dimensions of the floral spur (from its tip to the stigma), lateral sepals and lip of 10 randomly selected flowers from five plants were measured using a steel ruler. The standing crop of nectar in the floral spurs of flowers from 15 plants was sampled at 0900 with calibrated micropipettes to establish its volume. A Bellingham and Stanley 0–50% refractometer was used to establish the nectar sugar concentration. Samples of nectar from five flowers were spotted onto filter paper and analysed for constituent sugars using high pressure liquid chromatography, as detailed by Van Wyk (1993). Nectar of the closely related hawkmoth-pollinated taxon *Disa cooperi* Rchb.f was collected at a site near Underberg in KwaZulu-Natal and analysed for sugar constituents for purposes of comparison with *D. scullyi*.

2.3. Pollinator observations

A total of 40 h over six days spread across the four years of this study were spent carrying out pollinator observations. This included 3 h of evening observations using a flashlight to check for the possibility of visits by moths. Diurnal observations were typically carried out between 0900 and 1400, but also included one period of observations between 0600 and 0900. Pollinators were either observed during the foraging bouts to establish their behaviour or captured with handnets and pinned for later identification and measurement of the proboscis.

2.4. Pollination success

Pollination success was estimated in 1993 and 2005 from the proportion of flowers with pollen deposited on the stigmas and one or more pollinaria removed from the anther. One flower was randomly selected from each of 39 plants in 1993, while all of the open flowers ($n = 128$) on 28 plants were censused in 2005.

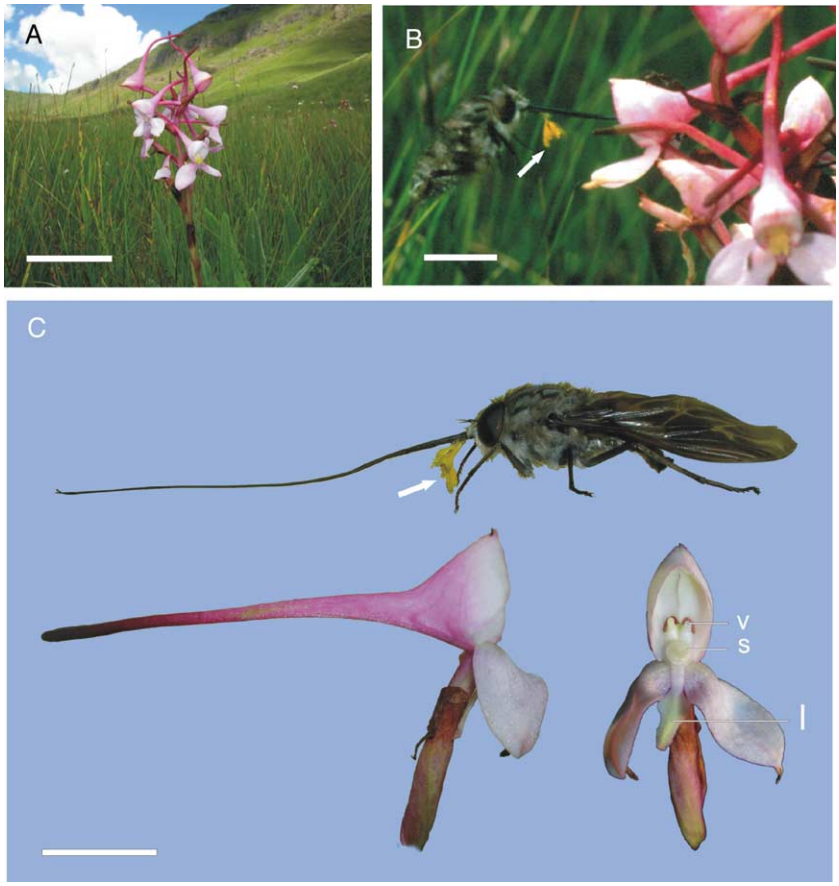

Fig. 1. (A) Inflorescence of *Disa scullyi* in grassland habitat in the Cape Drakensberg mountains. Scale bar (applicable to inflorescence): 50 mm; (B) long-proboscid fly *P. ganglbaueri* probing a flower of *D. scullyi*. Pollinaria (arrowed) are visible on its proboscis. Scale bar: 10 mm; (C) *P. ganglbaueri* positioned next to flowers of *D. scullyi*. Pollinaria (arrowed) are attached to the proboscis. The two entrances to the galea are visible in the front view of a *D. scullyi* flower. Abbreviations: v = viscidium, s = stigma, l = lip. Scale bar: 10 mm.

3. Results

3.1. Floral traits

The average floral spur length in the *D. scullyi* population was 42.0 mm (SD=2.45, $n=10$). The entrance to the spur is via two small apertures left by the petals which otherwise block most of the entrance to the galea (Fig. 1D). These apertures permit the entrance of a narrow insect proboscis while ensuring that the proboscis passes directly over and against the viscidia situated at the tip of the rostellum lobes. The slightly upturned orientation of the spurs (Fig. 1A, B) ensures that a hovering insect will press firmly down onto the rostellum lobes once its proboscis is fully inserted, thus leading to firm adhesion of the viscidia.

Only a very faint scent from the flowers of *D. scullyi* could be detected by the human nose. The average volume of nectar in the spurs was 3.4 μl (SD=1.4, $n=15$), while its sugar concentration by weight was 30.3% (SD=2.9, $n=15$). The nectar sugar composition was fructose (11%), glucose (14%) and sucrose (75%) for *D. scullyi*, and fructose (30%), glucose (23%) and sucrose (47%) for *D. cooperi*.

3.2. Pollinator observations

Insect visits to flowers of *D. scullyi* were infrequent. All of the observed visits were by the large long-proboscid fly *Prosoeca ganglbaueri* (Nemestrinidae). Four flies were directly observed between 0900 and 1200 while visiting plants in the population. Three of these flies were captured and two of them were found to carry loads of *D. scullyi* pollinaria attached to the basal underside of the proboscis. Prior to their capture, these flies visited up to 12 plants in a sequence and probed most of the open flowers on each plant. Each flower was probed for an estimated duration of c. 2–3 s. Pollinaria attached to the proboscis of these flies were observed to be dragged over the stigma as the flower is entered. In one case a fly was observed to remove several pollinaria from flowers as it foraged in the population. In addition, three flies carrying large loads of *D. scullyi* pollinaria were observed hovering next to rocks 50–100 m from the population and one of these was captured.

The average proboscis length for captured flies was 40.1 mm (range=38.5–41 mm, $n=4$), while the average number of pollinaria carried by these flies was 2.8 (range=0–6, $n=4$). A fly which was photographed, but not captured, carried four pollinaria (Fig. 1B).

3.3. Pollination success

Flowers of *D. scullyi* are not capable of autonomous self-pollination and thus rely on animal vectors for pollination. Flowers were well-visited in both census years. In 1993, 53.6% of the single flowers sampled from 38 plants had at least one pollinaria removed and 37.8% had been pollinated. In 2005, an average of 80.5% (SD=39.7) of open flowers on 28 plants had at least one pollinarium removed and 71.2% (SD=45.4) had been pollinated. As might be expected from the flower structure (see above), pollinaria appear to be removed independently, and not in pairs: flowers with only one pollinarium removed made up 28.9% of all flowers in 1993 and 30.8% of all flowers in 2003.

4. Discussion

The observations reported here indicate that flowers of *D. scullyi* are pollinated by the long-proboscid fly *P. ganglbaueri*. Six of the seven individuals of *P. ganglbaueri* captured or observed in the vicinity of the population carried pollinaria of the orchid. These were the only insects observed to pollinate the flowers during 40 h of diurnal and nocturnal observations spread over several years. The close morphological fit between fly and flower (the proboscis and spur lengths are almost an exact match), together with clear patterns of convergence in floral traits between *D. scullyi* and other plants in the *P. ganglbaueri* guild (Goldblatt and Manning, 2000; Goldblatt et al., 2001) suggests that further observations would not yield substantially different results.

P. ganglbaueri is a keystone pollinator species in the montane summer rainfall region of South Africa (Goldblatt and Manning, 2000). In the genus *Disa* alone, there are at least four species (*Disa oreophila*, *Disa amoena* H.P. Linder, *Disa nivea* H.P. Linder, and *Disa scullyi*) that depend on this fly for pollination (Johnson and Steiner, 1995, Goldblatt and Manning, 2000; Anderson et al., 2005).

Disa scullyi is a member of *Disa* section *Hircicornes,* a nectar-producing clade that includes the bee-pollinated *Disa versicolor* Rchb.f., long-proboscid fly pollinated *Disa rhodantha* Schltr. and hawkmoth-pollinated *D. cooperi* (Johnson et al., 1998). *Disa scullyi* and *D. cooperi* have a number of synapomorphic features, including a green lip, and on the basis of a cladistic analysis appear to be sister taxa (Linder and Kurzweil, 1999). In *D. cooperi* the lip is broad and scent-producing, while in *D. scullyi* it is narrow and very weakly scented. Morphological divergence in the lip may thus reflect an evolutionary shift between long-proboscid fly and hawkmoth pollination in these two species (Johnson, 1995).

Table 1
Comparison of *Disa scullyi* and its putative sister taxon *Disa cooperi*

	D. scullyi	*D. cooperi*
Pollinator	Long-proboscid flies	Hawkmoths
Spur length	c. 42 mm	c. 42 mm
Entrances to galea	Two	One
Scent production	Not detectable	Present (in the evening)
Flower colour	Mainly pink, some parts white	Mainly white, some parts pink
Lip width	c. 2 mm	c. 10 mm
Lateral sepal orientation	Faces forward	Reflexed
Nectar volume	c. 3.5 μl	c. 1.5 μl
Nectar concentration	c. 30%	c. 35%
Sucrose : hexose ratio in nectar	75 : 25	47 : 53

Data for *D. cooperi*, apart from the sucrose : hexose ratio, are taken from Johnson (1995).

Interestingly, the two species are otherwise very similar in most morphological features as well as nectar chemistry (Table 1), suggesting that modification of just a few traits, notably scent production, are required for a shift between a long-proboscid fly and hawkmoth pollination system. Populations of *D. scullyi* and *D. cooperi* tend to be found in different habitats (marshes and open grasslands, respectively) and are not known to form hybrids. It would, nevertheless, be interesting to determine whether pollinators discriminated strongly between the two species in mixed arrays. Recent molecular evidence presented by Archibald et al. (2004) suggests incomplete ethological isolation in sympatric populations of a hawkmoth- and long-proboscid fly-pollinated sister species pair in *Zaluzianksya* (Scrophulariaceae).

Shifts between hawkmoth and long-proboscid fly pollination have probably occurred often in the history of the southern African flora and represent an evolutionary "line of least resistance" (Stebbins, 1970) on account of the lack of necessity for profound changes in morphology and nectar properties (Table 1). Johnson et al. (2002), for example, demonstrated a shift from hawkmoth to long-proboscid fly pollination in *Zaluzianskya* (Scrophulariaceae) which involved mainly loss of scent production and some minor morphological changes. Reconstructing the history of pollination system shifts in *Disa* section *Hircicornes* will require more data on the pollination systems of species related to *D. scullyi* and *D. cooperi*, as well as a well-resolved species-level phylogeny.

Specialized mutualisms, such as those between long-proboscid flies and the plants they pollinate, may be vulnerable to failure as habitats change and populations of either mutualist partner become smaller. The larval habitat requirements for *P. ganglbaueri* are unfortunately not yet known. Adult flies, on the other hand, clearly require a guild of plants in order to meet their nectar requirements throughout the flight period. In the vicinity of the study site flies obtained nectar from the flowers of *Zaluzianskya microsiphon* (O. Kuntze) K. Schum (Scrophulariaceae), *Cycnium racemosum* Benth.(Scrophulariaceae), *Gladiolus oppositiflorus* Herbert (Iridaceae), and *D. scullyi*. As *Z. microsiphon* is by far the most important source of nectar for *P. ganglbaueri* in the Drakensberg mountains (Johnson et al., 2002; Anderson et al., 2005) it is likely that *D. scullyi* relies on this more common species to subsidize the nectar requirements of its pollinators.

Several populations of *D. scullyi* appear to have become extinct and the likely cause is either draining of the wetland habitat or heavy trampling by cattle (SD Johnson, personal observation). The study population does experience some cattle trampling (c. 10% of inflorescence were affected in this way), but occupies a habitat that is relatively intact on account of its isolation which makes intensive farming activities difficult. Interestingly, high levels of pollination success were recorded, especially in 2005 when over 70% of flowers were pollinated. This suggests that failure of the specialized mutualism is not occurring in this population.

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